United States Department of Agriculture
Food Safety and Inspection Service
Office of Public Health Science
Laboratory QA/QC Division
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Laboratory Guidebook
Notice of Change

Chapter **new**, revised, or archived: MLG 5A.00

Title: FSIS Procedure for the Use of *Escherichia coli* O157:H7 and O157:NM (Nonmotile) Screening Tests

Effective Date: 10/19/05

Description and purpose of change(s):

FSIS has validated use of a commercial PCR based screening procedure for raw ground beef and raw beef trim products. Procedures for performing alternative validated tests, both lateral flow devices, to be used if the primary test is unavailable are also included in this chapter. All samples identified as potentially positive for *Escherichia coli* O157:H7 by either primary or alternative tests are subject to cultural confirmation as described in this chapter and MLG 5 Detection, Isolation, and Identification of *Escherichia coli* O157:H7 and O157:NM (Nonmotile) from Meat Products.

Approved: Charles Pixley, 8/9/05

Procedure Outline

5A.1 Introduction

5A.1.1 General

This method describes the use of a commercial PCR-based screening procedure to screen test enrichment cultures of ground beef and beef trim products for *Escherichia coli* O157:H7 and O157:NM (Nonmotile). In the event that the BAX® system equipment or BAX® E. coli O157:H7 MP test kits are unavailable, this method describes the use of lateral flow devices that FSIS may use to screen for *Escherichia coli* O157:H7 and O157:NM.

Samples identified as potentially positive for the presence of *Escherichia coli* O157:H7 and O157:NM by any of these tests are subject to cultural confirmation as described in MLG 5.

Unless otherwise stated all measurements cited in this method have a tolerance of ± 2%.

5A1.2 Limits of Detection

For this method, *Escherichia coli* O157:H7 and O157:NM detection limits are determined to be better than 1 colony forming unit (cfu) in a 65g sample.

5A.2 Safety Precautions

E. coli O157:H7/NM is a human pathogen with a low infectious dose. (Ingestion of 100 cells can cause disease.) The use of gloves and eye protection is highly recommended and all work surfaces shall be disinfected prior to and immediately after use. Laboratory personnel must abide by CDC guidelines for manipulating Biosafety Class II pathogens. A Class II laminar flow biosafety cabinet is recommended for activities with potential for producing aerosols of pathogens. All available Material Safety Data Sheets (MSDS) should be obtained from the manufacturer for the media, chemicals, reagents and microorganisms used in the analysis. The personnel who will handle the materials should read all MSDS sheets.

5A.3 Quality Control Procedures

a. All media must be pre-warmed to 18-35ºC prior to use.

b. *E. coli* O157:H7 strain 465-97, or equivalent, shall be used as the positive control. See MLG 5, Section 5.3c for the positive culture control procedures.

c. *E. coli* ATCC strain 25922, or equivalent, shall be used as the negative control for bead capture and screen tests.

d. Prepare at least one "blank" (incubated but un-inoculated pre-enrichment/ enrichment broth) to provide a sterility control for the process.

5A.4 Equipment, Reagents and Media

In addition to equipment, reagents and media used in analysis of samples as described in MLG 5, the following materials will be needed.

a. PCR tube holder (Qualicon or equivalent)
b. Cell lysis tube cooling block (Qualicon or equivalent) held at 5 ± 3ºC
c. Heating block set at 37 ± 2ºC
d. Heating block set at 95 ± 2ºC
e. Repeating pipettor to deliver 200 ± 20 µL, and sterile tips
f. Pipettor to deliver 5 ± 1 µL, and sterile disposable filtered tips
g. Pipettor to deliver 150 ± 15 µL, and sterile disposable filtered tips
h. Eight-channel pipettor to deliver 50 ± 5 µL, and sterile disposable tips
i. 12 X 75 mm Falcon 352063, or equivalent, tubes

j. Cell lysis tubes and caps, cell lysis tube rack and box (Genemate 8 strip tubes, ISC Bioexpress, T-3120-5 or equivalent)
k. Pipettor and pipettes to deliver 5 mL
l. BAX® System PCR Assay for Screening *E. coli* O157:H7 MP kit (Qualicon # 17720673) held at 5 ± 3ºC
m. Optional for Lateral Flow Device Testing:

Transia™ Card *E. coli* O157:H7 (Diffchamb # ECO157), RapidChek® Pathogen Screening Test Kit (Strategic Diagnostics, Inc. # 7000160), or equivalent
Polypropylene tubes and sterile pipets
Heating block set at 97-100°C, boiling water bath, or autoclave (isotherm cycle)

5A.5 Sample Preparation and Primary Enrichment

Perform sample preparation and pre-enrichment as described in MLG 5, Section 5.5.

5A.6 The BAX® System and BAX® E. coli O157:H7 MP Test for Screening *Escherichia coli* O157:H7 and O157:NM (Nonmotile)

5A.6.1 Test Procedure

Follow the current BAX® System User's Guide for preparing reagents, performing the test, and reading the results. The equipment must be set up, operated, and all records documented according to laboratory work instructions.

5A.6.2 Interpretation of Results

a. Samples that test BAX®-negative shall be reported as negative. Cultural analysis shall continue as per MLG 5, Sections 5.6 and 5.7 for sample pre-enrichments that test BAX®- positive, indeterminate, or have a signal-error result. Or based on the findings of a cause analysis, the laboratory may analyze the indeterminate or signal error result samples by:

- repeating the BAX® analysis from the rack loading step
- preparing new BAX® tubes and repeating the analysis or
- screen testing with a lateral flow device (LFD) described in Section 5A.7.

If a LFD is utilized, the LFD result shall be reported.

b. In analytical runs where the positive control tests BAX® -negative, indeterminate, or has a signal-error result, the entire batch of samples is affected and a cause analysis shall be performed. Based on the findings the laboratory may analyze the samples by:

- repeating the BAX® analysis from the rack loading step
- preparing new BAX® tubes and repeating the analysis
- screen testing with a lateral flow device (LFD) described in Section 5A.7 or
- preparing fresh samples from reserve tissues

The results of the chosen test shall be reported.

5A.7 Use of Lateral Flow Devices for Screening *Escherichia coli* O157:H7 and O157:NM (Nonmotile)

In the event that the BAX® system equipment fails or BAX® E. coli O157:H7 MP test kits are unavailable, the following lateral flow devices may be used until the BAX® system is available to screen for *Escherichia coli* O157:H7 and O157:NM. Any Lateral Flow Devices used should meet or exceed the requirements for screen tests outlined in MLG chapter 5, section 5.4.3a. The laboratory should follow the manufacturer's guidelines or other validated laboratory procedures. **RapidChek**® and **Transia**™ lateral flow devices have been validated for use in FSIS laboratories.

5A.7.1 Test Procedure

a. Remove the appropriate number of test devices from storage.
b. Transfer 1-3 ml of the enrichment broth (from each Stomacher bag) into a polypropylene tube with a sterile pipette. For use with a heating block, use ~ 1 ml. Heat the capped tubes at 97-100ºC for the time specified by the manufacturer.
c. Follow the current manufacturers' instructions for performing the test.

5A.7.2 Interpretation of Results

Follow the current manufacturer's instructions for reading the test device results.

5A.8 Selected References

Centers for Disease Control and Prevention and National Institutes of Health (CDC/NIH). 1999. BioSafety in Microbiological and Biomedical Laboratories, 4th ed. U.S. Government Printing Office, Washington, D.C. also found at the CDC internet site.

BAX® System PCR Assay for Screening E. coli O157:H7 MP User Guide Supplement and Automated Detection for Bacterial Screening User Guide, Dupont Qualicon.

RapidChek® *E. coli* O157: (including H7) Test User Guide, Strategic Diagnostics Inc.

Transia™ Card *E. coli* O157 Manufacturer's Insert, Diffchamb